

AN AROMATIC ACID FRACTION OF CIGAR SMOKE CONDENSATE

S. OSMAN and J. BARSON*

Eastern Utilization Research and Development Division, Agricultural Research Service,
United States Department of Agriculture, Philadelphia, Pennsylvania 19118

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Abstract—The esterified acid fraction of cigar smoke condensate has been further fractionated according to the degree of unsaturation by column chromatography on silicic acid coated with silver nitrate. The following acids have been identified as the corresponding methyl esters by chromatographic and spectroscopic methods: benzoic, phenylacetic, 2-phenylpropionic, sorbic, and *m*- and *p*-toluic acid.

INTRODUCTION

THE acid fraction of tobacco leaf and smoke has been the subject of many investigations.¹ Quin and Hobbs² used gas chromatography to separate and characterize carboxylic acids in cigarette smoke, many of which are related to plant metabolism. A similar study of cigar smoke has also been reported.³ More recently publications⁴⁻⁶ from this laboratory described the variations of certain volatile acids in cigarettes of different tobacco types and in the smoke condensates therefrom.

In a continuing study of the constituents of cigar smoke⁷ we have further investigated the volatile acidic fraction therein. Previous characterization³ of the constituents in this fraction was based solely on gas chromatographic retention data. As with most studies on tobacco smoke composition, the complexity of the mixture was such that complete separation of the components of this fraction is extremely difficult, even with the more sensitive techniques for isolation and identification. In our present study we have attempted to improve the separation by further fractionating the acids prior to gas chromatography in order to obtain a more selective division into chemical types. Although many acids still remain to be identified, we now wish to report the isolation and identification of the following compounds from cigar smoke condensate: benzoic acid, sorbic acid, toluic acids (*m*-, *p*-), phenylacetic acid, and 2-phenylpropionic acid. Except for benzoic acid, this is the first report, to our knowledge, of the presence of these compounds in tobacco smoke, although other aromatic acids have been identified in cigarette smoke by Yang and Wender.⁸ We have also determined the amounts of these compounds in four cigar types containing unblended filler tobacco.

* Senior and Junior Research Fellows, respectively, Cigar Manufacturers Association of America.

¹ Tobacco Mfgs. Standing Committee Research Papers, No. 3, "The Constituents of Tobacco Smoke—An Annotated Bibliography" (Edited by H. R. Bentley and E. G. N. Berry). London (1959.)

² L. D. QUIN and M. E. HOBBS, *Analyt. Chem.* **30**, 1400 (1958).

³ I. SCHMELTZ and W. S. SCHLOTZHAUER, *Tobacco Sci.* **6**, 90 (1962).

⁴ I. Schmeltz, R. L. STEDMAN and R. L. MILLER, *J. Assoc. Offic. Agric. Chemists* **46**, 779 (1963).

⁵ R. L. STEDMAN, D. BURDICK and I. SCHMELTZ, *Tobacco Sci.* **7**, 166 (1963).

⁶ R. L. STEDMAN, D. BURDICK, W. J. CHAMBERLAIN and I. SCHMELTZ, *Tobacco Sci.* **8**, 79 (1964).

⁷ S. OSMAN and J. BARSON, *Phytochem.* **3**, 587 (1964).

⁸ C.-H. YANG and S. H. WENDER, *J. Chromatog.* **8**, 82 (1962).

RESULTS AND DISCUSSION

The acid fraction isolated from the smoke condensate according to the method described in the experimental section (basic extraction of the original ether extract, followed by acidification and re-extraction with ether) obviously will not include those compounds that partition more favorably into water, i.e. succinic acid, lactic acid, etc. Even with these omissions, the fraction is still a complex mixture. Figure 1 shows part of the chromatogram and most of the more than forty peaks present in the complete chromatogram obtained by programming an aliquot of the esterified acid fraction at 4° per min prior to column chromatography. The need for preliminary fractionation is clearly indicated. It has been our experience that any attempts at collecting eluates from such a complex mixture is not only awkward but inefficient in that mixtures giving complex and misleading spectra are obtained. Column chromatography on silicic acid coated with silver nitrate has been used successfully in the separation of olefinic esters⁹; we have applied this separation technique to the ester derivatives of the acid fraction in the hope that we would obtain more selective separation. Four main fractions

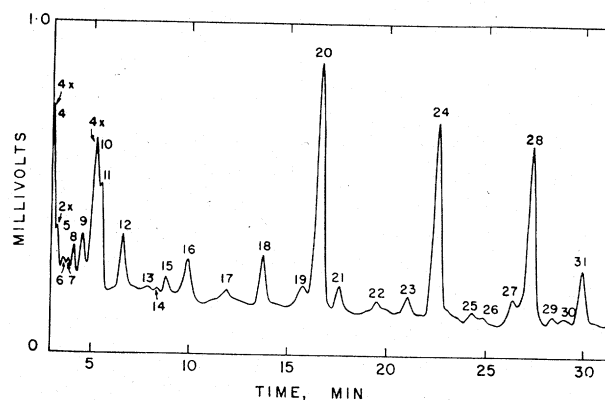


FIG. 1. GAS CHROMATOGRAM OF TOTAL ACID FRACTION (AS THEIR CORRESPONDING METHYL ESTERS) WITHOUT PRIOR COLUMN CHROMATOGRAPHIC SEPARATION.

were obtained by this method. Figures 2 and 3 are gas chromatograms of the first and second fractions, respectively. The third and fourth fractions, like the second, also contained one major component peak each. Collection of eluates of the second, third, and fourth fractions were made initially since it was suspected that fraction 1 contained esters of aliphatic acids previously studied. The second fraction corresponded to peaks 24 and 27 in Fig. 1. Peak 24 was identified as methyl benzoate by infrared and ultraviolet spectral analysis of the corresponding eluate and by cochromatography; peak 27, a doublet (Fig. 3), was tentatively identified as a mixture of *m*- and *p*-methyl toluate by u.v. analysis and cochromatography. The major component in the third fraction corresponded in retention time to peak 20 of Fig. 1 and had an i.r. spectrum almost identical with methyl sorbate although apparently extraneous peaks were noted at 3000 and 1480 cm^{-1} and 650 cm^{-1} in the unknown. These extraneous peaks may be due to the presence of trace amounts of benzene remaining from the column chromatographic separation. Ultraviolet spectral comparison and cochromatography confirmed the identity of the eluate as methyl sorbate. Sorbic acid is used in certain commercial cigar types as an antimicrobial agent which probably accounts for its presence

⁹ M. R. SUBBARAM and C. G. YOUNGS, *J. Am. Oil Chemists' Soc.* **41**, 150 (1964).

in the smoke condensate. Fraction four contained methyl phenylacetate and methyl 2-phenylpropionate (peaks 28 and 31 respectively in Fig. 1) as determined by i.r., u.v., and cochromatographic comparisons employed in the same manner as above.

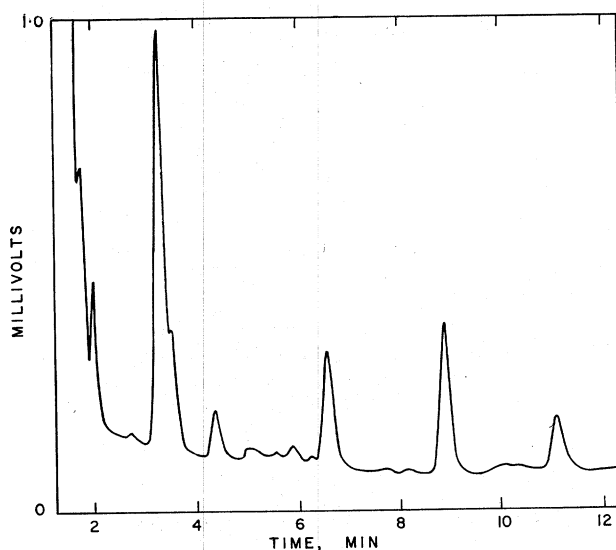


FIG. 2. GAS CHROMATOGRAM OF COLUMN CHROMATOGRAPHIC FRACTION 1 OF ACIDS (AS METHYL ESTERS).

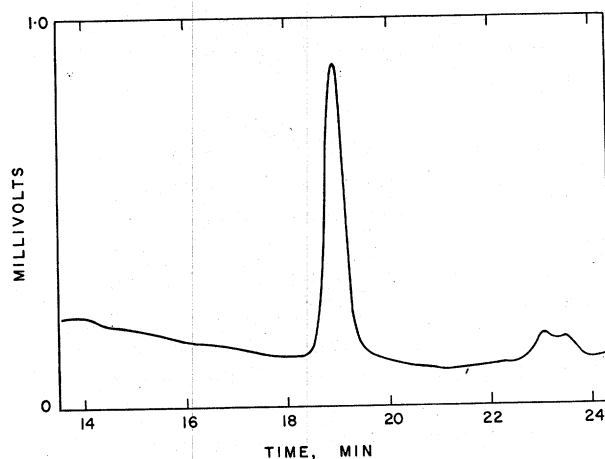


FIG. 3. GAS CHROMATOGRAM OF COLUMN CHROMATOGRAPHIC FRACTION 2 OF ACIDS (AS METHYL ESTERS).

Quantitative Analysis

Quantitative comparison of the contents of benzoic, phenylacetic, and 2-phenylpropionic acids in four cigar types are summarized in Table 1. Recoveries were about 60 per cent for the individual compounds with the greatest loss apparently occurring in the esterification step. Duplicate experiments with one of the cigar types gave results which indicate that large quantitative differences are detectable. Quantitative data for *m*- and *p*-toluic acid are not

reported because of the negligible amounts found in all the cigar types studied ($< 1 \mu\text{g}$ per cigar). No peak corresponding to sorbic acid was observed in the four types reported here (Table 1), which is expected since the samples were custom-made without preservative.

TABLE 1. AMOUNTS OF CERTAIN ACIDS PRESENT IN FOUR CIGAR SMOKE CONDENSATES

Compound	$\mu\text{g per cigar}^*$				
	1	2	3		4
			<i>a</i>	<i>b</i>	
Benzoate	25	17	23	19	9
Phenylacetate	11	14	20	23	5
2-Phenylpropionate	3	4	5	3	trace

* Uncorrected for losses in isolation. *a* and *b* are duplicate values. Cigars were perfectos, 130 mm length, and were smoked to an average butt length of 85 mm.

EXPERIMENTAL

The smoking apparatus and collection of the smoke condensate have been described in a previous publication.¹⁰ In order to obtain material for identification, 1500 cigars were smoked at an accelerated rate (a 2 sec puff every 25 sec instead of every minute). The cigars smoked in this phase of the work were mainly commercial types and several brands were used. For quantitative studies standard smoking conditions¹⁰ were used for collecting the condensates of four custom-made cigar types containing unblended filler. Twenty-five cigars were smoked for each analysis.

Isolation of the Acid Fraction from the Total Smoke Condensate

The condensate was removed from the traps by successive washings with diethyl ether and 0.1 N NaOH. The amounts of reagents used in this operation was variable and depended on how much was needed to thoroughly remove the condensate from the traps. The ether solution was then extracted with 0.1 N NaOH until the aqueous extract was basic. The aqueous solutions were then pooled and acidified in the cold with 2 N H₂SO₄ and the solution was extracted with ether. The ether solution of acidic material was dried over anhydrous magnesium sulfate for 24 hr and then concentrated *in vacuo* to a constant weight.

Esterification of the Acids Isolated from the Smoke Condensate

A methanol solution (100 ml) containing 12.5% boron trifluoride was added to the crude acid fraction (500 mg) and the mixture was refluxed for 1 hr. After cooling, an equal amount of water was added and the solution was extracted with light petrol (b.p. 60–70°). The hydrocarbon layer was washed with 0.1 N NaOH to remove unreacted acids and then with water until the aqueous washes were neutral. The petroleum ether solution was then dried over anhydrous magnesium sulfate. The dried solution was concentrated to approximately 1.0 ml by distillation on a spinning band column.

¹⁰ A. I. SCHEPARTZ, *Tobacco Sci.* 3, 144 (1959).

Column Chromatography of Esterified Acids

A column (10 × 1 in) containing silicic acid coated with AgNO₃ was prepared according to Subbaram and Youngs.⁹ The eluting solvents were: 150 ml of 15% benzene in light petrol; 100 ml of 50% benzene in petrol; 100 ml of 100% benzene, and 100 ml of 10% methanol in benzene. One hundred fractions (5 ml each) were collected using an automatic collecting device and the fractions were monitored by gas chromatography as described below.

Gas Chromatography of Ester Fractions

A Wilkens Aerograph Model A-350* equipped with thermal conductivity detection was used throughout the work. The column (5 ft × ¼ in) was packed with Wilkens FFAP Phase (18%) coated on acid-washed Chromosorb W treated with hexamethyldichlorosilane. The carrier gas (helium) flow rate was 60 ml per min at 80° and the detector and injector temperatures were 260°. For monitoring purposes the column temperature was programmed at 10° per min from 80° to 240°. In all other cases the programming rate was varied depending on the complexity of the solution to be chromatographed.

Identification of Chromatographic Peaks

The collection of chromatographic eluates has been described.⁷ Infrared spectra of the eluates were taken on the neat liquid contained in a Limit Research microcell (0.018 mm path length). Ultraviolet spectra were obtained on methanolic solutions. The spectrometers used were a Perkin Elmer Model 237 Infracord, and 202 ultraviolet spectrometer. Chromatography was done under the general conditions described above using a 4° per min programming rate for the column oven.

Quantitative Analyses

The amounts of the identified acids present in the condensate of four unblended cigar types was determined by gas chromatographic area-concentration relationships. The recovery of these acids in the isolation procedure was determined by adding known amounts of the acids to a fraction of the crude smoke condensate of one cigar type.

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* Mention of a specific commercial product does not constitute an endorsement by the U.S. Department of Agriculture over similar items not mentioned.